DAIDS

VIROLOGY MANUAL

FOR HIV LABORATORIES

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Compiled by

THE DIVISION OF AIDS

NATIONAL INSTITUTE OF ALLERGY & INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH

and

COLLABORATING INVESTIGATORS

HIV SYNCYTIUM-INDUCING (MT-2) ASSAY

I. PRINCIPLE

The MT-2 cell culture assay is used to detect syncytium-inducing (SI) variants of HIV. The presence of SI HIV variants has been associated with rapid progression to AIDS and a lower survival rate. The MT-2 assay is normally performed in duplicate, in 96-well flat-bottom cell culture plates. Other plate, tube or flask formats are permissible if the concentrations are adjusted accordingly.

MT-2 cells, an HTLV-I immortalized T-cell line, are cultivated with cell-free supernatants from HIV-infected PBMC cultures. Inoculated MT-2 cell cultures are monitored every 2-3 days, up to two weeks, for development of typical cytopathic effect (CPE), i.e.,, large ballooning syncytia.

II. SPECIMEN REQUIREMENTS

Supernatants from HIV-infected PBMC cultures are suitable specimens. Optimally, fresh coculture supernatants should be used, but flash-frozen supernatants are acceptable, provided they have been stored at -70^0C or lower and have not been subjected to freeze-thaw cycles. Any previously frozen supernatant should be recultured in PBMCs prior to or concurrent with the MT-2 assay to confirm viability.

III. REAGENTS

MT-2 cells: Human T-cells isolated from cord blood lymphocytes and cocultured with cells from patients with adult T-cell leukemia. (NIH AIDS Reagent Repository catalog #237).

MT-2 culture media: 500 mL RPMI 1640 supplemented with 50 mL fetal bovine serum, 10 mL penicillin (5000U/mL)/streptomycin (5000 µg/mL), and 5 mL 200 mM L-glutamine.

Positive control reference virus: NIH AIDS Reagent Repository catalog #629 (virus strain A018 or 018C).

IV. SUPPLIES AND EQUIPMENT

Sterile 96-well flat-bottom cell culture plate
Micropipettors to deliver 50, 150 and 200 µL
Sterile disposable pipette tips (Sterile ART tips may be used as an additional safeguard against contamination.)
Sterile centrifuge tubes
25 and 75 cm^2 cell culture flasks

2, 10 and 25 mL pipettes
Pipette-aid
Hemacytometer and cover slip
Inverted microscope
CO_2 incubator
Biological safety cabinet
Appropriate personal safety gear

V. PROCEDURE

A. Propagation of MT-2 cells

1. Thaw 2 vials of frozen MT-2 stock cells rapidly in 37^0C water bath.

2. Aseptically transfer cells to 15 mL centrifuge tube. Centrifuge at 600 x g for 10 minutes at room temperature. Remove DMSO-freeze media from the cells.

3. Resuspend the cell pellet in 10 mL MT-2 culture medium and transfer to a 25 cm^2 cell culture flask.

4. Incubate at 37^0C in 5% CO_2.

5. After 2 days incubation, measure cell number and viability. Split the cells when they number 10^6/mL. Seed 75 cm^2 cell culture flasks with 20 mLs at 5 x 10^5 viable cells/mL.

6. Incubate cultures at 37^0C in 5% CO_2. Split cells 1:3 every 3 to 4 days or 1:10 once each week.

B. MT-2 phenotype assay

1. Add 200 µL sterile PBS to each well designated "P" in the following diagram of a flat-bottomed 96 well cell culture plate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	P	P	P	P	P	P	P	P	P	P	P
B	P	NEG	1	P	2	P	3	P	4	P	POS	P
C	P	NEG	1	P	2	P	3	P	4	P	POS	P
D	P	P	P	P	P	P	P	P	P	P	P	P
E	P	P	P	P	P	P	P	P	P	P	P	P
F	P	NEG	5	P	6	P	7	P	8	P	POS	P
G	P	NEG	5	P	6	P	7	P	8	P	POS	P
H	P	P	P	P	P	P	P	P	P	P	P	P

2. Harvest MT-2 cells which are growing in the log phase. For the 96-well format, prepare a 4.0 mL suspension of 3.4×10^4 MT-2 cells/mL in MT-2 media.

3. Inoculate 150 µL of the MT-2 cell suspension into each well designated with a specimen number or "NEG" or "POS". (Based on the above diagram, twenty four wells will be seeded with MT-2 cells at a final concentration of 5×10^4 cells per well.)

4. Add 50 µL of MT-2 culture media to the wells designated "NEG". Add 50 µL of positive control virus to each of the wells designated "POS". Add 50 µL of HIV-infected culture supernatant to duplicate specimen wells (1 through 8). The final volume in each well will be 200 µL.

5. Incubate the plate at 37^0C in 5% CO_2. (If the incubator is not humidified, seal the plate in a ziplock bag, then incubate.)

6. On days 3, 6, 9, 12, and 14 (plus or minus 1), use an inverted microscope to examine each MT-2 well for syncytia formation. Positive syncytia formation is defined as 3 to 5 balloons or syncytia per well. Read carefully against the negative and positive controls wells. Document the first day of observed syncytia, e.g., "SI+d6" for an isolate which was first observed to produce syncytia on day 6.

7. After the wells have been examined, use a micropipettor and disposable tips to gentle resuspend the cells in each well and then remove 130 µL of suspension. Change tips between *every* well.

8. Refeed each well with 150 µL of MT-2 culture media. Return plate to ziplock bag, if needed, and continue incubation and observation.

9. Terminate the assay on day 14. If no syncytia are observed, score the isolate as nonsyncytium-inducing (NSI).

VI. QUALITY CONTROL

1. Positive virus control must produce observable syncytia within 7 days of incubation.

2. Negative control wells must not develop syncytia. Note: minor ballooning may occur as the MT-2 cells divide, but these "balloons" are significantly smaller than in the positive wells and are not true syncytia.

3. If either control does not react as expected, the assay is suspect and should be repeated.

VII. NOTES

False negative SI results, interpreted as "NSI", can result from non-viable virus supernatant. In cases when culture supernatant viability is unknown, a control PBMC culture should be performed.

False negative SI results can also occur if the MT-2 cells are compromised. Negative SI results should be confirmed if the positive SI control requires longer than 9 days to produce typical cytopathic effect, i.e., syncytia.

VIII. REFERENCES

Japour AJ, Fiscus S, Mayers D, Reichelderfer P, Kuritzkes D. A microtiter MT-2 syncytia assay. IX International Conference on AIDS, 1993. PO-B41-2484; vol I, page 549. Berlin, Germany.

Harada S, Koyanagi Y and Yamamoto N. Infection of HTLV-III/LAV in HTLV-1-carrying cells MT-2 and MT-4 and application in a plaque assay. Science **229**:563-566, 1985.

Tersmette M, Lange JM, deGoede RE, deWolf F, Eeftink-Schattenkerk JK, Schellenkens PT, Coutinho RA, Huisman JG, Goudsmi J and Miedema F. Association between biological

properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet **1:**983-985, 1989.

Koot M, Keet IP, Vos AH, deGoede RE, Ross MT, Coutinho RA, Miedema F, Schellenkens PT and Tersmette M. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann Intern Med **118:**681-688, 1993.

St. Clair MH, Hartigan PM, Andrews JC, Vavro CL, Simberkoff MS and Hamilton JD. Zidovudine resistance, syncytium-inducing phenotype, and HIV disease progression in a case-control study. The VA Cooperative Study Group. J Acquir Immune Deficiency Syndrome **6:**891-897, 1993.